ACTIVE NEMS ARRAYS FOR BIOCHEMICAL ANALYSES

CROSS-REFERENCE TO RELATED APPLICATION(S)

This application is based on U.S. Application No. 60/224,109, filed August 9, 2000, the disclosure of which is incorporated by reference.

FIELD OF THE INVENTION

This invention is generally directed to biofunctionalized nanoelectromechanical devices (BioNEMS) for enabling dynamical single-molecule force assays of solutions.

BACKGROUND OF THE INVENTION

The revolution in molecular biology provided by DNA cloning and sequencing techniques, X-ray crystallography and NMR spectroscopy has offered unprecedented insights into the molecules that underlie the life process. However, in contrast to the dramatic rate of progress in sequencing and structural approaches, there remain major stumbling blocks in applying modern molecular knowledge fully, as many of the analytical techniques presently available remain remarkably similar to those used in the relatively early days of molecular biology and biochemistry.

For example, conventional gel electrophoresis and "blotting" techniques for determining the presence and amount of a given messenger RNA (mRNA) in a cell requires vast quantities of cells (~10^9), and 2 days to complete. Even the most advanced DNA array chip techniques require ~$2x10^7$ cells. Accordingly, advances in fields ranging from molecular medicine and basic cell biology to environmental toxicology are being hampered by the bottleneck generated by the sensitivity and speed of these conventional analytical techniques.

A growing literature of chemical force microscopy (CFM) has shown that a modified Atomic Force Microscope (AFM) can be tailored to measure the binding force of interactions ranging from single hydrogen bonds and single receptor-ligand interactions to single covalent bonds. For example, an early study showed the force required to break a single hydrogen bond to be on the order of 10 pN and subsequent work enabled the direct measurement of receptor/ligand interactions

(~50-250 pN) and DNA hybridization (~65 pN - 1.5 nN). CFM has also been utilized to study conformational changes such as the deformation of the polysaccharide dextran by an applied force and have elucidated the unfolding of the protein titan (~100-300 pN). In addition to the above experiments performed with CFM, important advances have been made with optical tweezers. In particular, they have been used to study step-wise forces in biological motor motion and sub-pN polymer dynamics.

While the range of forces associated with many biochemical systems are well within the capability of AFM instrumentation to detect, there are severe limitations to the systems in which these devices can be used. For example, an AFM cantilever in solution does not have the temporal response characteristics needed to permit the binding and unbinding of biological ligands and their receptors to be followed reliably. Especially important are variation on the few μs timescale, characteristic of important classes of conformational changes in large biomolecules. High frequency response is also critical to following the stochastic nature of receptor ligand interaction. Most receptor-ligand pairs interact dynamically: binding, remaining engaged for times ranging from microseconds to seconds (depending on the exact receptor-ligand pair), and then releasing. The analysis of biomolecules is thus limited by both the vast quantities of materials required and the smearing in time inherent in even the most sensitive assays to date.

Perhaps even more significant is the substantial size of the equipment required for performing AFM/CFM, and the density limits imposed by optical detection of the probe motion. In addition, although the sensing mechanism is generally compact, even the so-called "lab on a chip" devices optical detectors are typically employed which require large, complicated support machinery, such as readers and sample preparation apparatus. These are not portable or easily reduced in size.

Third, optical tweezers employ diffraction-limited spots, hence the optical gradient forces generated are far too spatially-extended to permit direct manipulation of individual biomolecules under study. Instead, biofunctionalized dielectric beads typically having diameters in the range 0.1 to 1 μm, are used to adhere to the analytes. Accordingly, this technology is not readily scalable to nanometer dimensions or to large-scale integration.

Finally, all of the aforementioned techniques involve force sensors with active surface areas that are quite large compared to the molecular scale; hence it can be very difficult to achieve single-molecule sensing.

Accordingly, a need exists for a system and method for single molecule sensing in solution having higher sensitivity and temporal response with reduced overall size and active surface area.

SUMMARY OF THE INVENTION

The present invention is directed to a biofunctionalized nanoelectromechanical device (BioNEMS) for sensing single-molecules in solution. This can be accomplished in two distinct modes of operation. The first is "passive" and involves measuring the variation in the resonance motion of the BioNEMS device during a binding event. The second is "active" and involves driving the devices with an external signal and looking for changes in the response upon a molecular binding event. The molecular detector according to the invention generally comprises at least one nanomechanical resonator, a detector integral with the mechanical resonator for measuring the vibration of the resonator, and electronics connected to the detector for communicating the results to a user.

In one embodiment, the molecular detector comprises a solution reservoir which contains the solution to be tested, a biofunctionalized mechanical resonator arranged within the reservoir in fluid contact with the solution, and a detector integral with the resonator for detecting the resonance of the resonator. During operation, the Brownian fluctuations inherent in a non-turbulent solution drive random fluctuations in the position of the mechanical resonator. The spectral density of the solution-induced response will depend on the nature of the solution, i.e., viscosity, temperature, flow; and the geometry of and the material used to construct the mechanical resonator. A molecule binding out of solution onto the surface of the resonator will inherently change the mechanical properties of the resonator causing a variation in the response. The resonator is preferably biofunctionalized such that only specified molecules will bind thereto, such that a binding event indicates the presence of the specific molecule in the solution. The detector is engaged with the resonator to detect the response over time such that a change in the response can be measured to determine when a binding event occurs and multiple changes in the resonance can be monitored to determine the frequency

of binding events for a particular sample. The measurement of a resonance change can be used to determine the absolute presence of a particular molecule in a solution, and the frequency of binding events can be utilized to determine the concentration of the molecule in a particular solution.

Any mechanical resonator or device suitable to provide mechanical response in a solution may be utilized in the present invention, such as, for example, vibrational resonators, counter rotating and rotating resonators, torsional resonators, or compound resonators. For simplicity, all such putential mechanical detection devices will be hereafter referred to as "resonators". The resonator may be made from any suitable material, such as, for example, silicon oxide, silicon, silicon carbide and gallium arsenide. The resonator may have any physical properties suitable for detection of single-molecular binding events in solution. For example, the resonator may have a thickness between about 10nm and 1μm, a width between about 10nm and 1μm, and a length between about 1μm and 10 μm. The resonator may have a resonance motion vacuum frequency between about 0.1 and 12MHz. The resonator may have a force constant between about 0.1mN/m and 1 N/m. The resonator may have a Reynolds number between about 0.001 and 2.0. The resonator may have a mass loading coefficient between about 0.3 and 11. Finally, the resonator may have a force sensitivity of about 8fN/$\sqrt{}$Hz or greater.

In one embodiment of the invention, the mechanical resonator is a vibrating cantilever of simple or complex geometry. In such an embodiment, the cantilever is preferably a piezoresistive device such that the response is measured by sensing the voltage change in the cantilever over time. In such an embodiment, the molecular detector is preferably biofunctionalized with a ligand or receptor.

In another embodiment, the molecular detector further comprises a substrate disposed within the reservoir and adjacent to the resonator, where the substrate is biofunctionalized with a ligand capable of molecular interaction with the receptor, or vice-versa. Alternatively, the substrate may also be biofunctionalized with a receptor that is not capable of molecular interaction with the receptor on the resonator, but which is capable of molecular interaction with a ligand which itself is capable of molecular interaction with the receptor on the resonator.

In still another embodiment, the molecular detector comprises at least two resonators arranged adjacent to one another, wherein one of the resonators is biofunctionalized with a receptor to form a receptor resonator and at least one of the

resonators adjacent to the receptor resonator is biofunctionalized with a ligand capable of molecular interaction with the receptor such that the resonators can be coupled through the ligand/receptor functionalization.

In yet another embodiment, the molecular detector comprises at least two resonators arranged adjacent to one another, wherein at least one of the resonators is a driver resonator biofunctionalized with a receptor and having a driving element capable of resonating the driver resonator at a chosen frequency or frequencies, and at least one of the resonators adjacent to the driver resonator is biofunctionalized with a ligand capable of molecular interaction with the receptor on the driver resonator such that the resonators can be coupled through the ligand/receptor functionalization.

In still yet another embodiment, the molecular detector comprises at least three resonators, including, two driver resonators comprising driving elements capable of resonating the driver resonators at a chosen frequency in antiphase to each other, and a follower resonator disposed between the two driver resonators. In such an embodiment, at least one of the driver resonators is biofunctionalized with a receptor and the follower resonator is biofunctionalized with a ligand capable of molecular interaction with the receptor on the driver resonator such that the resonators can be coupled through the ligand/receptor functionalization. In such an embodiment, the driver may be any device suitable for driving the resonator at a specified frequency, such as, for example, a piezoresistive driver device.

In still yet another embodiment, the detector is integral with the resonator. Any detector suitable for detecting the response of the resonator may be utilized, such as, for example, a piezoresistive transducer or an optical detector. In an embodiment utilizing a piezoresistive transducer, the transducer may be made of p+ doped silicon.

In still yet another embodiment, the invention is directed to a system of molecular detectors as described above. In one such embodiment the molecular detector system comprises at least one microfluidic channel and at least one array of molecular detector devices disposed within the at least one microfluidic channel, wherein the array comprises a plurality of biofunctionalized nanometer-scale mechanical resonators and where each resonator has at least one detector for measuring the response motion of the resonator.

In still yet another embodiment, the invention is directed to a method of utilizing a molecular detector as described above. In one such embodiment the method of detecting a molecule of interest comprises providing a molecular detector comprising a biofunctionalized nano-scale resonator. Placing the molecular detector into a solution such that the resonator moves based on the thermal motion of the solution and such that in the presence of a species capable of molecular interaction with the biofunctionalized resonator the response of the resonator is restricted, and measuring the response of the resonator such that a change in the response of the resonator is communicated to a user.

In still yet another embodiment, the invention is directed to a method of manufacturing a molecular detector as described above. In one such embodiment the method of manufacturing the molecular detector comprises supplying a substrate, depositing a photoresist on the substrate, exposing a pattern comprising the resonator on the photoresist, etching the substrate to form the resonator, and removing the photoresist.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features and advantages of the present invention will be better understood by reference to the following detailed description when considered in conjunction with the accompanying drawings wherein:

FIG. 1 is a schematic depiction of a first embodiment of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 2 is a schematic depiction of the operation of the first embodiment of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 3a is a schematic depiction of a second embodiment of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 3b is a schematic depiction of a third embodiment of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 3c is a schematic depiction of a fourth embodiment of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 3d is a schematic depiction of a fifth embodiment of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 3e is a schematic depiction of a sixth embodiment of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 3f is a schematic depiction of a seventh embodiment of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 4 is a pictorial depiction of exemplary mechanical resonators according to the present invention.

FIG. 5 is a schematic diagram of a conventional surface-etching technique for producing a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 6 is a pictorial depiction of a prototype of a biofunctionalized nanoelectromechanical sensing device according to an exemplary embodiment of the present invention.

FIG. 7 is a graphical representation of the detection properties of a prototype of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 8 is a graphical representation of the detection properties of a prototype of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 9 is a graphical representation of the detection properties of a prototype of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 10 is a graphical representation of the detection properties of a prototype of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 11 is a graphical representation of the detection properties of a prototype of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 12 is a graphical representation of the detection properties of a prototype of a biofunctionalized nanoelectromechanical sensing device according to the present invention.

FIG. 13 is a schematic depiction of a second embodiment of a system of biofunctionalized nanoelectromechanical sensing devices according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

A biofunctionalized nanoelectromechanical device (BioNEMS) capable of sensing single-molecules in solution by measuring the variation in the resonance motion of a BioNEMS resonator device during a binding event is described herein. The biofunctionalized nanoelectromechanical device according to the invention being henceforth referred to as a molecular detector.

The molecular detector **10** according to one embodiment of the invention is shown schematically in FIGs. 1 and 2 and comprises a solution reservoir **12** containing a solution **14** having at least one biofunctionalized nanoelectromechanical resonator **16** arranged therein. A detector **18** in signal communication with an electronic signal processor **20** is attached integrally to the resonator **16** such that any movement by the resonator **16** is measured by the detector **18** amplified and transmitted to the processor **20**.

During operation, as shown in FIG. 2, the thermal fluctuations or Brownian motion inherent in the solution **14** create mechanical displacement **22** of the position of the mechanical resonator **16**, while simultaneously the presence of the solution **14** around the resonator **16** produces a dampening force on the resonance motion of the resonator **16**. In the case of the vibrational cantilever resonator **16** shown in FIGs. 1 and 2, the Brownian movement of the molecules in the solution **14** create a mechanical displacement of the free end of the resonator **16**. The dynamic properties of this solution-induced displacement or response **22** depends on the nature of the solution **14**, i.e., viscosity, temperature, flow; and the geometry of and the material used to construct the mechanical resonator **16**. Although the thermal buffeting and solution dampening of the resonator **16** makes conventional resonance detection techniques associated with AFM difficult to perform, molecules **24** binding out of solution **14** onto the surface of the resonator **16** change the mechanical properties of the resonator **16** causing a variation or restriction in the thermally induced resonance **22** and this restriction is then sensed by the detector **18** amplified and communicated to the processor **20**. To ensure that the detector **18** only registers the presence of specified molecules of interest, the surface of the resonator **16** may be biofunctionalized or modified such that only specified molecules will bind thereto. For example, in FIGs. 1 and 2, the resonator **16** has been biofunctionalized with a ligand **26** chosen such that only a specified receptor molecule **24** will bind thereto. Such a modification, allows for the detection of

minute quantities of specific molecules in the solution **14b** utilizing the detector **10** according to the current invention.

Table 1, below displays a list of physical characteristics of a series of typical simple vibrational cantilever resonators according to FIGs. 1 and 2.

Table 1: Characteristics of Simple Vibrational Cantilever Resonators

#	Thickness (t)	Width (w)	Length (l)	Vac. Freq. MHz	Force Constant (k) mN/m	$\Re$	Mass Loading Coeff.
1	100nm	1μm	10μm	1.2	39	1.884	3.37
2	30nm	300nm	3μm	4.1	12	0.5793	3.37
3	30nm	100nm	3μm	4.1	3.9	0.0644	1.12
4	10nm	300nm	3μm	1.4	0.43	0.1978	10.11
5	10nm	100nm	3μm	1.4	0.14	0.0220	3.37
6	10nm	100nm	1μm	12	3.9	0.1884	3.37
7	10nm	30nm	1μm	12	1.2	0.0170	1.01
8	10nm	10nm	1μm	12	0.40	0.0019	0.34

Although a simple single resonator **16** single ligand biofunctionalized **26** detector **10** is shown in FIGs. 1 and 2, any combination of resonators **16** and biofunctionalization can be utilized to create detectors **10** having unique assay properties. Examples of some exemplary molecular detectors **10** according to the current invention are shown in FIGs. 3a to 3f, and discussed below.

FIG. 3a shows a molecular detector **10** comprising a single resonator **16** with a ligand biofunctionalization **26'** and a substrate **28** with a receptor biofunctionalization **26"** designed to assay for either the presence of a free receptor or free ligand in solution or to assay for compounds that stabilize or compete with the interaction between the functional ligand/receptor. As shown, the resonator **16** will be tethered to the substrate **28** when the ligand **26'** and receptor **26"** interact such that the mechanical response **22** of the resonator **16** is strongly restricted.

FIG. 3b shows a molecular detector **10** comprising a single resonator **16** with a receptor biofunctionalization **26'** and a substrate **28** with a second receptor

biofunctionalization **26"** designed to assay for molecules **24** that contain target recognition sites for both receptors **26'** and **26"** on the same molecule.

FIG. 3c shows a molecular detector **10** comprising multiple resonators **16** with a simple receptor biofunctionalization **26** designed to assay for single molecules **24**, in which the ligand molecules **24** in the solution **14** have been modified with star dendromers **30** such that the binding of the ligand molecule **24** to the receptor biofunctionalization **26** more greatly alters the viscous drag, and therefore the mechanical response **22** of the resonator **16**. Although star dendromer modifiers **30** are shown in this embodiment, any modifier which would enhance the resonator/solution coupling to provide sensitivity enhancement to the molecular detector **10** may also be utilized.

FIG. 3d shows a molecular detector **10** comprising multiple coupled resonators **16** with a receptor biofunctionalization **26'** on one resonator **16'** and a ligand biofunctionalization **26"** on an adjacent resonator **16"** such that the motion of the resonators **16'** and **16"** is coupled through the ligand/receptor biofunctionalization and such that the motion of both resonators is monitored simultaneously. In this embodiment, the correlation of the motion of the two resonators **16'** and **16"** allows for greater noise reduction, increasing the sensitivity of the molecular detector **10**. This molecular detector **10** could be designed to assay for compounds that either bind with or stabilize or compete with the functional ligand/receptor interactions between the adjacent resonators.

FIG. 3e shows a molecular detector **10** comprising at least two different resonators: a driver resonator **16a** and a follower resonator **16b**. As in the embodiment shown in FIG. 3d, a receptor biofunctionalization **26'** is provided on the driver resonator **16a** and a ligand biofunctionalization **26"** is provided on the adjacent follower resonator **16b** such that the motion of the resonators **16a** and **16b** is coupled through the ligand/receptor biofunctionalization and such that the motion of both resonators **16a** and **16b** is monitored simultaneously. However, in the embodiment shown in FIG. 3e a driver (not shown), actuated piezoelectrically, thermoelastically or by other physical mechanisms, actively drives the motion of the driver resonator **16a** such that the motion **22** is tuned to the most sensitive amplitude and frequency possible for the geometry of the driver resonator **16a**. The correlated motion of the driver resonator **16a** and follower resonator **16b** are then monitored to detect whether the ligand/receptor pair are functionally linked. A

molecular detector **10** of this design could then be utilized to assay for compounds that either bind with or stabilize or compete with the functional ligand/receptor interactions between the adjacent resonators.

FIG. 3f shows a molecular detector **10** comprising at least three different resonators: a (+) driver resonator **16a**, a (-) driver resonator **16b** and a follower resonator **16c**. As in the embodiment shown in FIG. 3e, a receptor biofunctionalization **26'** is provided on one of the driver resonators **16a** and a ligand biofunctionalization **26"** on the adjacent follower resonator **16c** such that the motion of the resonators **16a** and **16c** is coupled through the ligand/receptor biofunctionalization and such that the motion of both resonators **16a** and **16c** is simultaneously monitored. As in the embodiment shown in FIG. 3e a piezoelectric driver (not shown) actively drives the resonance motion of the driver resonators **16a** and **16b** such that the motion is tuned to the most sensitive amplitude and frequency possible for the resonator geometry. The correlated motion of the driver resonator **16a** and follower resonator **16c** are then monitored to detect whether the ligand/receptor pair are functionally linked. However, in the actively driven embodiment shown in FIG. 3e, hydrodynamic coupling between the resonators **16a** and **16c** may limit the dynamic range of the molecular detector **10**. Providing a second active resonator **16b**, operated in antiphase, nulls the hydrodynamic coupling, thereby improving the signal/noise of the molecular detector **10** thus produced. A molecular detector **10** of this design could then be utilized to assay for compounds that either bind with or stabilize or compete with the functional ligand/receptor interactions between the adjacent resonators. There may be advantages to configuring multiple-driver geometries (beyond the pair of drivers described here) to provide more refined schemes for nulling the background fluidic coupling to the "detector" cantilever.

Although the embodiments of the molecular detectors **10** discussed above in relation to FIGs. 1 to 3 all describe a single molecule ligand/receptor biofunctionalization **26**, it will be understood that any suitable biofunctionalization **26** may be utilized in the current invention, such as, DNA hybridization, chemical bonds and protein unfolding. For example, the molecular detector may by biofunctionalized to screen the products of combinatorial chemistry, or to profile gene expression in cells, or to sense the concentrations of growth factors, hormones and intracellular messengers in cell biology, or to yield

information about specific blood chemistry, or as a general physiology sensor, or as a detector for exposure to pathogens or toxins either in the environment or in a patient. Likewise, although all of the exemplary embodiments shown in FIGs. 1 to 3 all show single biofunctionalized sites **26** on the resonators **16**, any method of biofunctionalization or number of biofunctionalized sites may be utilized on the resonators **16** of the current invention.

Although the embodiments of the resonator **16**, shown in FIGs. 1 to 3 are all depicted as simple vibrational cantilever resonators **16**, it should be understood that any NEMS construct capable of resonance motion under the thermal or Brownian motion of the solution **14**, wherein the resonance is sufficiently sensitive to allow detection of a restriction in the resonance motion **22** caused by a single molecule binding event can be utilized in the present invention. FIG. 4 shows pictorial representations of several different conventional NEMS resonators **16** suitable for use in the current invention, such as, for example, rotational resonators, torsional resonators and composite resonators. In addition, it should be understood that although the resonators described above are all macrodevices, resonators comprising single molecules coupled to a substrate may be utilized according to the present invention such that the molecule itself would be modified to interact with a molecule of choice in a solution.

The present invention is also directed to a method of manufacturing the BioNEMS molecular detector **10**. FIG. 5, shows a schematic diagram of an exemplary technique for manufacturing a BioNEMS resonator **16** according to the present invention utilizing surface-etching. There are two parts to manufacturing the resonator **16** of the present invention utilizing a NEMS manufacturing method; the actual manufacturing process, and the mask design. FIG. 5, shows one embodiment of the method for making the resonator **16** according to the present invention, including the number of photolithographic steps required, and how the resonator **16** is separated from the substrate. The basic sequence, as shown, include: (a) examining and cleaning a starting substrate comprising, in the embodiment shown, three layers, a structural layer **32,** a sacrificial layer **34** and a substrate layer **36**; (b) modifying the surface of the structural layer **32** to form the resonator **16** via an electron beam mask **38** and depositing the photoresist and pattern resist etch metal for the resonator **16**; (c) etching the pattern into the structural and sacrificial layers **32** and **34**; and (d) etching the sacrificial layer **34**

to undercut the resonator **16** to free the resonator **16**. Although this embodiment only shows an etching process which undercuts the sacrificial layer **34**, it should be understood that additional etching may be performed to create deeper undercuts and/or etching of the substrate **36** below such that insulation between the resonator **16** and the substrate **36** is increased.

While the above embodiment exemplifies a method for forming the resonator **14** of the present invention utilizing a conventional NEMS process, any manufacturing process suitable for forming the nanometer resonator **16**, such as, for example, wafer bonding and etch-back may be utilized. In the wafer bonding and etch-back process a silicon wafer substrate has a very thick oxide layer deposited or thermally grown on the surface. This thick oxide layer is then covered by a thin silicon nitride layer. The resonator **16** is deposited and fabricated on this silicon nitride layer. The surface of the resonator **16** is then covered by resist, and the back of the substrate **36** is removed chemically leaving only a "frame" to support the devices. When utilizing this approach, the resonator **16** is preferably not close to the substrate **36**.

The resonator **16** can be fabricated utilizing any suitable substrate material, such as, for example, silicon. In a preferred embodiment, a single-crystal silicon substrate is utilized for the resonator **16**. Other silicon materials may also be utilized to make the resonator **16** of the present invention, such as, for example, thick epitaxial silicon on single crystal wafers with highly doped layers as leads, or polycrystalline silicon. Although the manufacturing process described above describes the surface nanomachining of a silicon-based material, the resonator **16** of the current invention can be made of any material suitable for surface nanomachining, capable of biofunctionalization and inert to chemical modification by and of the molecules **24** in the solution **14**. Examples of conventional nanomachining materials suitable for use in the current invention include: silicon-based systems, such as silicon oxide (SOI) or silicon carbide and gallium-arsenide-based systems (GaAs). Other substrate materials may be used, as well, including insulating materials such as diamond and quartz thin films.

Any detector **18** suitable for detecting the resonance motion of the resonator **16** in solution may be utilized in the molecular detector **10** of the current invention. For example, the detector **18** may comprise vibrational or strain sensitive devices integrally connected to the resonator **16**, as shown in FIGs. 1

and 2. In one exemplary embodiment the detector **18** is a piezoresistive strain transducer, as shown in FIG. 1. In this embodiment the transducer detector **18** converts the motion of the resonator **16** into an electrical signal via the strain-induced change in resistance of a conducting path on the top surface of the resonator **16**. These resistance changes are then amplified and communicated to a processor **20** designed to provide a read-out of the signal changes. Although the detector **18** may be made of any suitable material, in one embodiment it is made from a p+ doped silicon epilayer formed on the top surface of the resonator **16**.

Although only strain-type transducer detectors are described above, any detector suitable to monitor the motion of the resonator **16** on a time-scale suitable for monitoring the biomolecular interactions of interest may be utilized. For example, the detector **18** may also comprise an externally mounted device, such as, an optical-laser, fluorescence based position sensor, electromagnetic or magnetic.

The signal monitor system and processor **20** for any of the above detection schemes can comprise any suitable digital signal processor capable of measuring the signal change from the detector **18** and transmitting that information to the user, such as, for example, a printed circuit board having a pre-amplifier, an AD converter and driver circuit, and a programmable chip for instrumentation specific software; or a multichip module comprising those elements.

Regardless of the specific embodiment of the molecular detector **10** utilized, all operate on the principle that a BioNEMS resonator will inherently posses a large thermally driven motion or mechanical response when disposed within a solution due to the repeated interaction between the resonator and the molecules of the solution, and that a chemical bond between the functionalized portion of the resonator and the molecule of interest will produce a detectable alteration of the mechanical response.

FIG. 6 shows a prototype notched cantilever resonator **16** utilized to test the sensitivity of molecular detectors **10** made according to the present invention. First, the theoretical force sensitivity of the molecular detector **10** was calculated and then the actual performance of a series of detectors utilizing the resonator shown in FIG. 6 was tested.

Table 2, below, summarizes the physical parameters for three prototypical notched cantilever resonator **16** according to FIG. 6. Utilizing the cantilever

resonator prototypes listed in Table 2 the physical properties of the molecular detector of the current invention were calculated.

Table 2: Characteristics of Notched Vibrational Cantilever Resonators

#	(t)	(w)	(l)	(l_1)	(b)	$\omega_0/2\pi$	K
1	130nm	2.5μm	15μm	2.5μm	0.6μm	0.51MHz	34mN/m
2	130nm	300nm	10μm	2.0μm	100nm	1.3MHz	20mN/m
3	30nm	100nm	3μm	0.6μm	33nm	3.4MHz	3.0mN/m

Because the resonator **16** is large compared to the size of the molecules **24** in the solution **14**, the thermal motion of the resonator **16** in solution **14** may be modeled in terms of stochastic forces, which are Markovian (because the time scale of the molecular collisions with the resonator are short compared to the frequencies of the macroscopic resonance motion of the resonator), and Gaussian (because the macroscopic motion is formed by a large number of molecular collisions). Accordingly, the resonance motion of the resonator **16** in the solution **14**, in its fundamental mode, can be described and modeled by the fluctuation-dissipation theorem.

Any suitable calculation can be utilized to estimate this dissipation, such as, simplified geometric model estimations, low Reynolds number fluid solution calculations, or experimental measurements. The stochastic motion (x) of the resonator **16** may then be found by solving its dynamical equation with an additional fluctuating force with the spectral density. For resonators at the submicron scale in solution, as in the present invention, dissipation is dominated by the viscous motion of the fluid driven by the vibration of the resonator **16**.

Because the size of the resonator **16** is much larger than the size of the individual molecules **24** in the solution **14** colliding therewith, an approximation of the force on each small section of the resonator **16** as a result of the solution **14** impinging thereon is equal to the force of the solution **14** acting on the length of an infinite beam with the same cross-section and velocity.

In the example of a single rectangular vibrational cantilever resonator **16** as shown in FIG. 1, the loading of the resonator **16** can be approximated by the Stokes equation for a cylinder according to EQ. 1, below.

$$L(\omega) = \frac{\pi \rho_L w^2}{4} \Gamma(\Re) \qquad (1)$$

where the prefactor is simply the volume displaced by the resonator **16**, while the function Γ, which depends solely on the Reynolds number (ℜ), must be calculated from the motion of the solution **14**. In this approximation, the fluidic forces from the solution **14** at each frequency and on each section of the resonator **14** are proportional to the displacement at that point.

Alternatively, a more complete calculation of the resonance motion of a resonator can be made utilizing the basic equations of motion. In the case of a notched vibrating cantilever resonator **16**, as shown in FIG. 6, the equation of motion for the displacement (x) at the end of the resonator **16** is that of a simple vibrating cantilever in vacuum according to:

$$\left|\tilde{x}\right| = \frac{\left|\tilde{F}\right|}{\left\{\left[K - \omega^2 M_{eff}(\omega)\right]^2 + \omega^2 \gamma_{eff}^2(\omega)\right\}^{1/2}} \qquad (2)$$

where x describes the motion of the free end of the cantilever resonator **16,** F is the applied force, K is a force constant dependent on the geometry of a resonator **16** of width (w), thickness (t) and length (l). EQ. 2 provides a complete description of the resonator's **16** resonance response both to the externally applied forces and, through the fluctuation-dissipation theorem, to the stochastic forces imparted from the solution **14**.

For a notched cantilever, as shown in FIG. 6, the force constant could be found according to the equation:

$$K = \frac{Et^3}{4l^3/w + \left(2l_1^3 - 6ll_1^2 + 6l^2 l_1\right)\left(\frac{1}{b} - \frac{2}{w}\right)} \qquad (3)$$

where (w) is the width of the end of the resonator **16**, (l) is the length of the resonator **16**, (t) is the thickness of the resonator **16**, (b) is the width of the notch legs **30** of the resonator **16**, and (l_1) is the length of the notched portion **32** of the resonator **16**.

The equations of motion for the resonator **16** are complicated because of the presence of a dynamic solution **14** surrounding and influencing the motion of the resonator **16**. Accordingly, in solution M_{eff} is the effective mass of the cantilever resonator **16**, which is dependent on the fluid loading of the solution **14**. In vacuum the effective mass follows the equation:

$$M_{eff} \cong \alpha \rho_c wtl\left[1+\frac{\pi}{4}\bar{T}\,\mathrm{Re}\{\Gamma\}\right] \tag{4}$$

which itself is dependent on the fluidic mass loading coefficient $\bar{T}$ according to:

$$\bar{T} = \alpha \rho_L w/\left(\rho_c t\right) \tag{5}$$

with ρ_L, ρ_C the density of the solution and resonator, respectively. As a result, thin resonators experience relatively large fluid loading (where $\rho_L/\rho_C = 2$, $\bar{T}$ ranges from 1 to 5). The value of Re{Γ} is unity for large $\Re$, is around 4 at $\Re$ equals 1, and continues to increase as $\Re$ decreases. Hence, for a value of w/t equal to 2, the mass loading factor is at least 5 at $\Re$ equal 1, and increases for proportionally thinner beams and lower Reynolds numbers.

In turn, γ_{eff} is the effective fluidic damping coefficient, according to EQ. 5, below.

$$\gamma_{eff} \cong \alpha \frac{\pi \rho_L}{4} w^2 l\left[\omega\ \mathrm{Im}\{\Gamma\}\right] \tag{6}$$

The parameter α relates the mean square displacement along the beam to the displacement at its end. For the fundamental mode of a simple rectangular vibrational cantilever resonator **16**, as shown in FIG. 1, $\alpha = 0.243$. In comparison, the notched vibrational cantilever resonator **16**, shown in FIG 6, $\alpha = 0.333$.

In addition, the term Γ corresponds to the fluidic coupling between the resonator **16** and the solution fluid **14** according to:

$$\Gamma(\Re) = 1+\frac{4iK_1\left(-i\sqrt{i\Re}\right)}{\sqrt{i\Re}K_0\left(-i\sqrt{i\Re}\right)} \tag{7}$$

where the Reynolds number ($\Re$) is given by the equation:

$$\Re(\omega) = \omega w^2/(4\nu) \tag{8}$$

where v is the kinematic viscosity of water and is equal to 1.022×10^{-6} m^2/s at 293 K.

Accordingly, for frequencies below ~ 1 MHz with resonators having a width less than or equal to 1 μm, the Reynolds number is less than or equal to 1.6. Thus, the damping of the resonator **16** arising from the motion of the solution **14** fluid is most dependent on the dimensions of the resonator **16** transverse to the resonance motion, e.g., in the case of a vibrational cantilever as shown in FIG. 1, the width and length of the resonator. This analysis indicates that with uniform scaling down of *all* dimensions, w,t,l $\propto$ d, the damping of a resonator **16** in solution **14** decreases as d with decreasing size of the resonator **16**, increasing the sensitivity of the molecular detector **10**.

In Table 3, below, a list of the calculated properties of the prototype notched vibrational cantilever resonators **16**, as shown in FIG. 6, are provided.

Table 3: Characteristics of Notched Vibrational Cantilever Resonators

#	t (nm)	w (nm)	l (μm)	l_1 (μm)	(b) (nm)	$\omega_0/2\pi$ (MHz)	K (mN/m)	$\Re$	$\bar{T}$
1	130	2,500	15	2.5	0.6	0.51	34	5.0	8.22
2	130	300	10	2.0	100	1.3	20	0.19	0.986
3	30	100	3	0.6	33	3.4	3.0	0.054	1.42

As described above, the thermal noise component arises, as described by the fluctuation-dissipation theorem, from the fluidic damping of the cantilever. The mechanical Q of these structures is approximated using the equation:

$$Q \sim \frac{\omega M_{eff}}{\gamma_{eff}} \sim \frac{\mathrm{Re}\{\Gamma(\Re)\}}{\mathrm{Im}\{\Gamma(\Re)\}} \qquad (9)$$

where fluid mass is assumed to dominate. It will be recognized that this expression is mostly independent of frequency, varying only over the range $0.2 < Q < 0.9$ as the Reynolds number ($\Re$) changes from 10^{-3} to 1. As described above, and as expected from the calculations, the mechanical Q of these resonators **16** in the solution **14** is much less than 1, whereas their W's in vacuum are typically of on the order of 10^4. Hence the fluidic dissipation resulting from the surrounding solution **14** completely determines the resonance **22** of the resonator **16**.

To quantitatively determine the effective force sensitivity of the resonator **16** and ultimately the molecular detector **10** described by the above equations of motion, the force acting on the resonator **16** from the thermal or Brownian motion of the solution **14** must be taken into account. With this regard, the minimum detectable force is defined according to:

$$F_{\min}(\omega / \omega_0) = \left[S_F(\omega / \omega_0)\right]^{1/2} \tag{10}$$

where the minimum detectable force (F_{min}) is defined by the force (S_F) acting on the resonator **16** as the result of the molecular motion of the molecules in solution **14**. This stochastic force acting on the resonator **16** can be directly related to the dissipative coefficient appearing in EQ. 2, such that the force spectral density is given by the Nyquist formula:

$$S_F = 4k_B T\gamma_{eff} \tag{11}$$

where k_B is Boltzmann's constant and T is the temperature of the solution **14**.

Likewise, the displacement fluctuations (S_x) are defined by the mechanical responsivity to the spectral force (S_F), according to:

$$S_x^{(\gamma)}(\omega) = S_F^{(\gamma)}(\omega) R_{mech}^{s}(\omega) \tag{12}$$

where the mechanical responsivity R_{mech} having units m/N is defined according to EQ. 13, below.

$$R_{mech} = \sqrt{R(\omega / \omega_0)} / K \tag{13}$$

where $R(\omega/\omega_0)$ is provided in analogy with Hooke's Law, -1/K = x/F:

$$R(f / f_0) \equiv \frac{K^2\left|\tilde{x}\right|^2}{\left|\tilde{F}\right|^2} = \left[\left\{\frac{\omega^2}{4\omega_0^2}\left(1+\frac{\pi}{4}\bar{T}\,\mathrm{Re}\left\{\Gamma\left[\Re\left(\frac{\omega}{\omega_0}R_0\right)\right]\right\}\right)-1\right\}^2+\left(\frac{\pi}{16}\bar{T}\frac{\omega^2}{\omega_0^2}\mathrm{Im}\left\{\Gamma\left[\Re\left(\frac{\omega}{\omega_0}R_0\right)\right]\right\}\right)^2\right]^{-1} \tag{14}$$

In FIG. 7, the response function $R(\omega/\omega_0)$, for three different vibrational cantilever geometries is provided. It is apparent from the plot that a finite frequency peak is present in the response function of the solution damped vibrational cantilever resonators.

As described in the previous section, the frequency dependent displacement spectral density and mean square response functions obtained in the presence of fluid coupling allow an estimation of the force sensitivity attainable for different resonator geometries. However, to determine the effective force sensitivity for the molecular detector **10** according to the present invention it is also necessary to determine the noise induced by the detector **18** or the electrical noise of the system. In the three notched-vibrational cantilever resonator molecular detector prototypes **10** shown in FIG. 6 and described above, a strain sensitive piezoelectric transducer **18** was utilized to detect the resonance motion of the resonator **16**. Accordingly, three additional terms are added to the real system force noise equation according to EQ. 15, below.

$$\left[S_F\right]_{eff} = \frac{1}{R_{mech}^2}\left\{\left[S_x\right]_{fluidic} + \frac{1}{R_{\det ector}^2}\left(\left[S_V^{out}\right]_{\det ector}^{RTO} + \left[S_V^{A}\right]_{amplifier}^{RTI}\right)\right\} \qquad (15)$$

In this equation S_F is equivalent to the spectral force or the force fluctuations applied to the resonator **16**, S_X is equal to the fluid-coupled noise of the resonator **16**, S_V^{out} is equal to the noise generated by the detector **18**, and S_{VA} is equal to the noise generated by the amplifier and other processor electronics **20**.

In the case of the prototype S_V^{out} arises from the thermal noise of the piezoresistive transducer where S_V^{out} is equal to:

$$S_V^{out} = 4k_BTR_T \qquad (16)$$

while S_{VA} arises from the readout amplifier's voltage and current noise according to:

$$S_{VA} = S_V + S_1R_T^2 \qquad (17)$$

where S_V and S_1 are the spectral density of the amplifier's voltage and current noise respectively.

In those cases where the response extends down to low frequencies, a third term must also be considered, the 1/f noise ($S_{1/f}$) in the transducer. Although this term must be considered, there is a fundamental difference between the 1/f noise and that of the fluid-induce displacement fluctuations. As such, in a preferred embodiment a lock-in detection scheme is used to measure the resistance such that

only the portion of the 1/f spectrum within the detection window will contribute to the noise. Alternatively, by probing the resistance at frequencies above the 1/f knee, this source of noise can be practically eliminated .

In contrast, the fluid-induced displacement fluctuation noise leads to changes in the resistance of the resonator that are within the detectable range regardless of the frequency probe current used. Hence, the entire noise spectrum from dc up to the frequency of the low pass filter is relevant.

The force sensitivity of the molecular detector **10** of the current invention, then, hinges on the maximum level of current bias that is tolerable, given that the responsivity is proportional to the bias current (R = IG), where the gauge factor (G) is equal to:

$$G = \frac{\partial R_T}{\partial x} = \frac{3\beta\pi_l(2l - l_1)R_T}{2bt^2} \qquad (18)$$

and where the parameter π_l is the piezoresistive coefficient of the p+ transducer material. The factor β accounts for the decrease in G due to the finite thickness of the of the conducting layer; β approaches unity as the carriers become confined to a surface layer of infinitesimal thickness.

To quantify some of the parameters for the prototype notched vibrational cantilever resonators shown in FIG. 6, the resonance motion and resistance of the resonators was measured. FIG. 8 shows the measured room temperature fundamental resonance motion for the first prototype cantilever resonator listed in Table 2 in vacuum. FIG. 9 shows a plot of the displacement of the prototype cantilever shown in FIG. 6 caused by the resonance motion versus resistance.

These plots yield a direct measurement of $G = 3x10^7$. For epilayers such as those used in the prototype molecular detectors shown in FIG. 6, the EQ. 18 yields a calculated value of $\beta = 0.7$ and $G = 6x10^8$ Ω/m. For the transducer geometry pictured in FIG. 6, a two-terminal (equilibrium) resistance of $R_T = 15.6k\Omega$ is obtained..

Using the values for the resistance and the gauge factor (G) above, it is possible to determine the maximum current bias, which is found by determining the maximum temperature rise deemed acceptable for the biofunctionalization disposed along the resonator. The geometry of the prototype devices shown in FIG. 6 causes dissipation to occur predominantly within the constriction regions (of width b). A

rough estimate of the heat loss to the surrounding solution may be obtained through the relationship:

$$\kappa_{Si} A \frac{\partial^2 T}{\partial x^2} = \kappa_{H_2O} P \nabla_n T \tag{19}$$

where P is the perimeter around cross-sectional area A of the resonator. Estimating that:

$$\nabla_n \sim T / w \tag{20}$$

and that,

$$\frac{\partial^2 T}{\partial x^2} \sim \frac{2(w+t)\kappa_{H_2O}}{\kappa_{Si} t w^2} \tag{21}$$

where $\kappa_{Si} = 1.48\text{x}10^2$ W/mK is the thermal conductivity of silicon and $\kappa_{H20} = 0.607$ W/mK is the thermal conductivity of water. In the dissipative region $x < l_1$,

$$2\kappa_{Si} tb \frac{\partial^2 T}{\partial x^2} \sim -I^2 R + (b+t)\frac{T}{b}\kappa_{H_2O} \tag{22}$$

where as boundary conditions, the temperature is continuous at l_1, as is the heat flux; and $\delta T/\delta x = 0$ at $x = l$.

This simple thermal conductance calculation indicates that, for example, a 1 K rise a the biofunctionalized tip is attained with a steady-state bias current of 250μA, leading to a power dissipation of roughly 10670μW. The maximal temperature rise of 12K occurs within the constricted region, approximately 2.3 μm from the support. For this bias current, the prototype molecular detector **10** yields a responsivity of R = IG ~ 8μV/nm.

Utilizing these parameters, an estimated coupled force sensitivity can be determined. For cantilever 1, assuming that a 1K rise at the tip is tolerable, the transducer-induced displacement noise is found to be $\sqrt{S_{VT}}/R = 1.8\text{x}10^{-12}$ m/√Hz. For a typical low noise readout amplifier with voltage and current noise levels of ~4nV/√Hz and ~5fA/√Hz, respectively (typical for JFET input low noise amplifiers) these same parameters yield an amplifier term $\sqrt{S_{VA}}/R = 4.4\text{x}10^{-13}$ m/√Hz.

To demonstrate the effects of scaling the resonator downward in size, cantilever resonators 2 and 3, having a geometry identical to that of cantilever resonator 1, are also considered. Utilizing the physical dimensions of cantilever 2

the above equations yields an R_T = 67kΩ and a G = 7.4x10_9 Ω/m. For cantilever resonator 2, assuming an 0.05K temperature rise at the tip of the resonator is tolerable yields a transducer-induced displacement noise $\sqrt{S_{VT}}/R = 6.3x10^{-14}$ m/√Hz and a readout amplifier contribution of $\sqrt{S_{VA}}/R = 8.0x10^{-15}$ m/√Hz. For cantilever resonator 3, the above equations yields an R_T = 258kΩ and a G = 7.39x10_{10} Ω/m. Again assuming an 0.05K temperature rise at the tip of the resonator is tolerable yields a transducer-induced displacement noise $\sqrt{S_{VT}}/R = 3.8x10^{-14}$ m/√Hz and a readout amplifier contribution of $\sqrt{S_{VA}}/R = 3.3x10^{-15}$ m/√Hz.

In FIGs. 10 to 12 the coupled force sensitivity per unit bandwidth calculations for the three prototype notched vibrational cantilever resonators 1 to 3 in Tables 2 and 3 utilizing three different detector bias currents are plotted verse the thermal force noise of the solution. These calculations include the combined noise from fluidic, transducer, and readout amplifier sources.

FIG. 10 shows that for a temperature rise of 1K at the resonator tip, even the largest resonator (cantilever 1) yields a remarkably low coupled force sensitivity $[S_f^{(c)}]^{1/2} \leq$ 85fN/√Hz for frequencies below 100KHz. This indicates that a molecular detector utilizing the cantilever 1 resonator would be capable of taking dynamical measurements on the ~10μs scale for absolute forces on the level of < 30 pN without averaging.

FIG. 11, shows that for an 0.05K temperature rise at the tip of the resonator the cantilever 2 resonator device yields even better force sensitivity, $[S_f^{(c)}]^{1/2} \leq$ 20fN/√Hz for frequencies below 0.5MHz (10% above the fluidic fluctuation limit). This indicates that a molecular detector utilizing the cantilever 2 resonator would be capable of taking dynamical measurements on the ~2μs scale for absolute forces on the level of < 15 pN without averaging.

Finally, FIG. 12, shows the attainable force sensitivity for a device utilizing a cantilever 3 resonator. Again, for an 0.05K temperature rise at the tip of the resonator the cantilever 3 resonator device yields a force sensitivity of $[S_f^{(c)}]^{1/2} \leq$ 10fN/√Hz for frequencies below 2MHz (10% above the fluidic fluctuation limit) and the force sensitivity rises to just ~11fN/√Hz for frequencies ≤3MHz. This indicates that a molecular detector utilizing the cantilever 2 resonator would be capable of taking dynamical measurements on the ~300ns scale for absolute forces on the level of < 20 pN without averaging.

Accordingly, the achievable coupled sensitivity for the molecular detector described herein, as low as ~8fN/√Hz, is limited predominantly by the fluidic fluctuations of the solution. As shown in Table 4, below, this threshold detection limit is well below the interaction forces of interest in most biological and chemical processes.

Table 4: Interaction Forces

Nature of Interaction	Interaction Force
Receptor/Ligand Interaction	50-250pN
Avidin-Biotin	90-260pN
Antibody-Antigen	50-300pn
Cadherin-Cadherin	35-55pN
DNA Hybridization	65pN-1.5nN
Chemical Bond	1-10nN
Covalent (C-C, C-O, C-N)	4.0-4.5nN
Covalent (Au-S, Si-C)	1-3nN
H-bond	10pN
Unfolding Forces	100-300pN
Protein (Titin) unfolding	150-300pN
Dexran bond twists	100-300pN

Although only molecular detectors **10** having single resonator assemblies **16** are shown in the Figures and discussed in the text above, the molecular detector **10** according to the present invention may also comprise a large array or system of resonator assemblies. One exemplary embodiment of such a system is shown schematically in FIG. 13, which shows a multiple channel array **40** of molecular detectors **10**, in which the array channels **42** are aligned in parallel on a single substrate **44** such that multiple or parallel processing of molecular samples can be carried out at one time. In this embodiment, multiple molecular detectors **10** are utilized for analysis of the molecules. It should be understood that while parallel and single array channels **42** are shown in FIG. 13, any suitable alternative geometry of channels **42** may be utilized such as, for example, folded channels may

be used to increase the length of the detector path without increasing the size of the array body **40**. Although the embodiment shown in FIG. 13 discloses a multi-channel array **40** in which the detector channels **42** are separated by walls **46**, the multi-channel detector array **40** could alternatively comprise a single "sheet" of detector arrays without walls between the channels **42**.

Further, while all of the resonators **16** of the molecular detector array system **40** could be functionalized to monitor for a single substance, as described in the previous embodiments, thereby providing greatly enhanced detector sensitivity, the resonators **16** of the detector array **40** system shown in FIG. 13 may also comprise individually biofunctionalized resonators such that multiple substances can be identified and monitored simultaneously. In addition, any combination of the various resonator embodiments shown and discussed in relation to FIGs. 3a to 3f, above, may be utilized in the molecular detector array system of the present invention.

Although specific embodiments are disclosed herein, it is expected that persons skilled in the art can and will design alternative molecular detectors, methods to produce the molecular detectors and/or molecular detector systems that are within the scope of the following claims either literally or under the Doctrine of Equivalents.